MOLECULAR DIAGNOSTICS SYSTEM AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of U.S. application Ser. No. 12/308,307, which is a U.S. national stage application of PCT/US2005/046772, filed on Dec. 21, 2005, which claims the benefit of U.S. provisional Application No. 60/638,177 filed Dec. 23, 2004. Each of these applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to an integrated nucleic acid test cartridge capable of performing extraction, amplification and detection together. It also relates to devices and methods for nucleic acid extraction alone, or extraction and amplification combined. Furthermore, it relates to devices and methods for amplification and detection combined. The cartridge may be equipped with a sensing means including enabling optical and electrochemical detection methods and it may also be equipped with a wax or absorbent filter extraction feature to separate target nucleic acid from the sample. The cartridge can perform various methods of amplification including polymerase chain reaction, rolling circle amplification and strand displacement amplification. The present invention also addresses novel amplification methods and reagents comprising single modified primers or pairs of modified primers, depending on the selected amplification method. Furthermore the present invention provides for integrated electrophoretic separation for primers from amplicons during a nucleic acid test.

BACKGROUND OF THE INVENTION

General Background on the Value of Nucleic Acid Testing

[0003] Applications of nucleic acid testing are broad. The majority of current commercial testing relates to infectious diseases including Chlamydia, gonorrhea, hepatitis and human immunodeficiency virus (HIV) viral load; genetic diseases including cystic fibrosis; coagulation and hematology factors including hemochromatosis; and cancer including genes for breast cancer. Other areas of interest include cardiovascular diseases and drug resistance screening, termed pharmacogenomics. The majority of testing currently occurs in centralized laboratories using non-portable and operationally complex instruments. Presently, tests generally require highly skilled individuals to perform the assays. As a result, the time taken between obtaining a sample suspected of containing a specific nucleic acid fragment and determining its presence or absence is often several hours and even days. However, as with other kinds of blood tests, physicians and others often require results more quickly and obtainable in a convenient user-friendly format. Consequently, there is a need for a portable analysis system capable of performing nucleic acid testing quickly and conveniently. A discussion of prior art relating to various aspects of nucleic acid testing is provided in the following sections.

Methods to Characterize Genetic Information

[0004] The clinical manifestation of a particular genetic characteristic can be different with different types or classes of genetic based diseases. This translates into different approaches to measure the genetic characteristic including SNP mutation detection, gene copy mutations and gene overexpression mutations. For example, some diseases such as hemochromatosis, cystic fibrosis or the oncogene p53, have one or a few very specific mutations which affect only a specific nucleotide. Considering hemochromatosis, there are two specific mutations. The clinical manifestation of this disease is an accumulation of iron in various tissues, which can be fatal if untreated. The most prevalent mutation is the G to A transition at nucleotide **845** in the gene, also known as (C282Y). See OMIM: Online Mendelian Inheritance in Man database, which can be found at the U.S. National Center for Biologic Information internet site. The second most prevalent mutation in the same hemochromatosis gene is a C to G transversion in exon 2, known as H63D. These are known as single nucleotide polymorphisms (SNPs). As every individual has two copies of each gene, the possible combinations of these genes are two wild type (homozygous wild type), two mutated genes (homozygous mutant) or one wild type and one mutated gene (heterozygous). In the case of hemochromatosis, individuals who are homozygous mutant exhibit the disease state, heterozygous individuals can be susceptible for some aspects of the disease as they accumulate higher levels of iron than do homozygous wildtype individuals. Also, for the purpose of determining if an individual is a carrier of the disease to their offspring, the ability to determine that an individual is heterozygous can be useful.

[0005] As a result, in testing for a genetic disease like hemochromatosis, it is useful to be able to have at least four analytical means or channels for detection. Here, one channel detects the presence of wild type C282, a second channel detects the presence of the mutant Y282 gene, a third channel detects the presence of the wildtype H63 gene and the fourth channel detects the presence of the mutant D63 gene. FIG. **12** provides a table of possible outcomes from a hemochromatosis test of this type and shows that it is possible to differentiate between homozygous or heterozygous, and that homozygous channels generate roughly twice the level of expression and thus signal in the test. Note that it is also useful to have one or more additional channels to use as positive and negative controls.

[0006] Some genetic mutations include multiple copies of the gene being present in the genome, causing a disease state in a patient. As an example the oncogene ZNF217 mapped within 20q13.2 has been found in multiple copies in individuals with colon cancer (Rooney et al., 2004, J. Pathol. Vol 204(3):282). Genetic triplication of the alpha-synuclein gene (SNCA) has been reported to cause hereditary early-onset Parkinsonism with dementia (Chartier-Harlin et al., 2004, Lancet, vol 364(9440):1167). Yamashita et al., 2004, European Neurology, vol 52(2): 101., have found that there is an increase in adult-onset Type III spinal muscular atrophy related to increased gene copies of the survival motor neuron (SMN2) gene. These gene copy mutations can be detected by using one or more required genes, such as the housekeeping genes (e.g. actin or glyceraldehyde-3-phosphate dehydrogenase). Overexpression mutations typically generate increased levels of mRNA and these can be detected.

Methods and Apparatuses for Extraction of Nucleic Acid

[0007] Nucleic acids found in cells can be deoxyribonucleic acid or ribonucleic acid and can be genomic DNA, extrachromosomal DNA (e.g. plasmids and episomes), mito-